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14. ABSTRACT

The ordering of collagen fibers within a tumor has significant influence on tumor metastasis: in murine breast tumor models, tumor cells move towards blood vessels along fibers that are visible via second harmonic generation (SHG), and SHG is exquisitely sensitive to molecular ordering. Tumor cells that are moving along SHG-producing (i.e. ordered) collagen fibers move significantly faster than those cells that are moving independently of SHG-producing fibers, and the extent of SHG-associated tumor cell motility is correlated with metastatic ability of the tumor model. Furthermore, the tumor-host interface of murine breast tumor models is characterized by radially oriented SHG-producing fibers associated with tumor cells invading the surrounding tissue. Consequently we believe that the process of establishing ordered fibers offers an exciting, and currently unexploited, therapeutic target. To take advantage of this, we must first learn the cellular players and molecular signals by which collagen ordering is induced. Therefore, in this application we propose to determine the key cells and signals which influence the ordering of collagen in breast tumors, determine if this ordering is predictive of metastasis, and develop new optical tools to study this ordering.

15. SUBJECT TERMS
Microscopy, metastasis

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Introduction.

The extent and nature of the ordering of collagen fibers within a tumor has significant influence on the process of tumor metastasis: in murine breast tumor models, tumor cells move towards blood vessels along fibers that are visible via second harmonic generation (SHG), and SHG is exquisitely sensitive to molecular ordering. Tumor cells that are moving along SHG-producing (i.e. ordered) collagen fibers move significantly faster than those cells that are moving independently of SHG-producing fibers, and the extent of SHG-associated tumor cell motility is correlated with metastatic ability of the tumor model. Furthermore, the tumor-host interface of murine breast tumor models is characterized by radially oriented SHG-producing fibers associated with tumor cells invading the surrounding tissue. Lastly, we have shown that treatment of tumors with the hormone relaxin, known to alter metastatic ability, alters the collagen ordering as detectable by SHG.

As locomotion along ordered (SHG-producing) fibers plays a pivotal role in the metastatic process, we believe that the process of establishing ordered fibers offers an exciting, and currently unexploited, therapeutic target. To take advantage of this, we must first learn the cellular players and molecular signals by which collagen ordering is induced. Therefore, in this application we propose to *determine the key cells and signals which influence the ordering of collagen in breast tumors*. We will do this by disrupting candidate cells and signals in mouse models of breast cancer using SHG-based measures of collagen ordering, and metastasis, as readouts. Additionally, we will *determine if SHG measures of collagen ordering in breast tumors are clinically useful predictors of metastatic outcome in breast cancer patient biopsies*.

This work will have great impact for several reasons. It will provide important insight into the molecular and cellular mechanisms by which the collagen in breast tumors is ordered, and how this ordering affects metastatic ability. In future work we can then exploit these findings by developing and evaluating clinically useful therapeutic techniques that will target, for the first time, the ordering of tumor collagen and hence attempt to inhibit metastatic ability, improving patient survival. This project will also explore whether collagen ordering in the tumor, as quantified by SHG, is a clinically viable predictor of metastatic outcome in patient biopsies. A measure of metastatic ability is extremely exciting, because there is currently an identified, pressing need for patient stratification based upon metastatic risk, in order to minimize 'over treatment' of patients who only require local therapy after resection, not systemic chemotherapy. This would improve patients' quality of life. Hence, this project has promise to be clinically relevant through two separate paths.

Keywords: Collagen, Metastasis, Second Harmonic Generation, Nonlinear Microscopy

Overall Project Summary:

The Statement of Work for this grant proposal was as described below. In summary, we were interested in exploring the cellular and signaling mechanisms underlying collagen ordering in breast tumors, understanding the impact this ordering has on metastasis, and determining if optical signatures (affected by this ordering) could be used to predict metastatic outcome in the clinical setting:

Specific Aim 1. Determine the role of macrophages in governing collagen ordering in tumors, and their mechanism of action. (Months 1-30)

1a) Modulate the presence of macrophages, then evaluate the effects on collagen ordering in tumors, and the effects on metastatic burden. (Months 1-12) Uses liposome treatment. ~50 mice. Verifies involvement of macrophages' in collagen ordering in tumors, the exact nature of their particular impact on collagen ordering, and the impact on metastasis.

1b) Manipulate the expression of candidate genes in macrophages, and evaluate the effects on collagen ordering in tumors, and the effects on metastatic burden (Months 13-30) Uses bone marrow transfer after irradiation. Source animals are one of 7 knockouts, for 7 candidate genes. ~50x7= 350 mice. Produces identity of key signaling molecules involved in collagen ordering in tumors, the exact nature of their particular impact on collagen ordering, and the impact on metastasis.

Specific Aim 2. Determine the role of Th1, Th2, and Tregs in governing collagen ordering in tumors, and their mechanism of action. (Months 31-60)

2a) Modulate the presence of each cell type, then evaluate the effects on collagen ordering in tumors, and the effects on metastatic burden.(Months 31-42) Uses cell transfer after antibody treatment. ~3x50=150 mice. Produces identity of key cells involved in collagen ordering in tumors, the exact nature of their particular impact on collagen ordering, and the impact on metastasis.

2b) Manipulate the expression of candidate genes in those cell types found significant in 2a, and evaluate the effects on collagen ordering in tumors, and the effects on metastatic burden (Months 43-60) Uses cell transfer after antibody treatment. Source animals are one of 7 knockouts, for 7 candidate genes. ~3x50x7=1050 mice. Produces identity of key signaling molecules involved in collagen ordering in tumors, the exact nature of their particular impact on collagen ordering, and the impact on metastasis.

Specific Aim 3. Determine if collagen ordering is a clinically useful predictor of metastatic ability in human tissue samples (Months 1-60).

1a) In archival specimens from breast tumors we will evaluate the predictive relationships between collagen ordering and metastatic outcome (Months 1-60). Uses pathology samples of 4 breast tumor types to determine if SHG can predict metastatic outcome. ~4x50=200 samples. Produces an assessment of SHG's predictive ability.

We have explored stromal effects of macrophages in the E0771 murine mammary adenocarcinoma grown in the mammary fat pad. In **(Burke R, et al. 2013)** we describe how ablation of stromal TNF-α as well as tumor associated macrophages altered collagen microstructure as quantified with SHG, and altered metastatic outcome. Looking downstream, in **(Perry S, et al. 2013)** we found that ablation of stromal matrix metalloproteinase 13 (MMP-13) altered collagen microstructure as measured by SHG, and altered metastatic outcome. We further found that ablating T-cells altered SHG-based measures of collagen microstructure in the primary tumor (N=7, 9, $p<0.05$) and increased the number of lung metastasis although the increase was not statistically significant (N=7,9, p=0.08) (Figure 1).

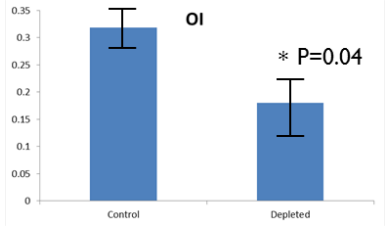
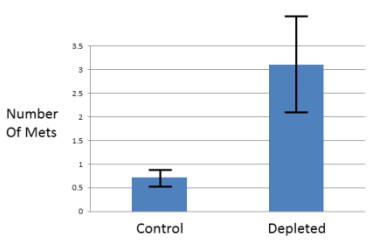
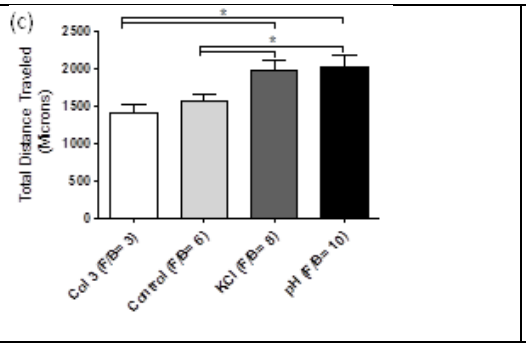


Figure 1. A (left). Depletion of T cells induces a significant alteration in the Order Index, an SHG-based measure of collagen order. **B** (right) T cell depletion also induces a significant increase in lung metastasis.



In each of the above, SHG measures of microstructure and metastatic outcome was altered. However, the underlying mechanism of the microstructure/metastasis relationship was unclear: This relationship may be entirely due to collagen microstructure's ability to alter cell motility. Alternatively, it may be entirely due to the influence of an upstream actor within the tumor which influences collagen microstructure and tumor cell motility separately. Or it might be a combination of these mechanisms. Therefore we explored the relationship between collagen microstructure (as quantified with SHG) and tumor cell motility in a relatively "clean" collagen gel system, in which confounding upstream actors are not present. The SHG measure of microstructure we used was the scattering directionality, i.e.the amount of SHG scattered in the forward direction (of laser propagation) divided by the amount scattered in the backwards direction, the F/B ratio. We developed optically thin collagen gels with different F/Bs and found that tumor cell motility was altered by the collagen gel F/B ratio (Figure 2). This suggests that the observed relationships between SHG measures of collagen microstructure and metastatic outcome are due, at least in part, to a direct influence of microstructure on cell motility.



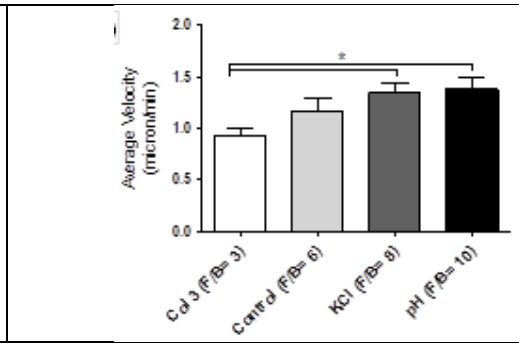


Figure 2. The average distance traveled by 4T1 tumor cells **(a, left)** was affected by gel F/B (ANOVA $p<0.05$). Post hoc analysis revealed the distance traveled in the two highest F/B gel categories was significantly higher than in the two lowest category. The average velocity of 4T1 tumor cells **(b, right)** was affected by gel F/B (ANOVA $p<0.05$). Post hoc analysis revealed that the average velocity in the two highest F/B gel categories was significantly higher than in the lowest category.

Extending these observations into clinical samples, in **(Burke K, et al. 2013)** we demonstrate that the SHG F/B ratio, a measure of collagen ordering, predicted lymphatic metastatic burden in patent breast cancer samples, and also revealed several intriguing relationships between stromal collagen ordering and tumor type, stage, and grade. These relationships between F/B and stage, grade, etc., were specifically between F/B and the stage/grade of the primary tumor *at the time the patient presented themselves to the clinic*. Based upon the clear relationship between breast tumor F/B and N stage (lymphatic metastasis) at that time of presentation, as shown in (Burke K, et al. 2013), we wondered if F/B could be used in patients who did NOT have evident metastasis at time of presentation. In other words could it predict the appearance of *future* metastasis?

In ER+ invasive ductal carcinoma (IDC) F/B is a significant predictor of duration of metastasis-free survival, based upon ten year followup data (K-M Log Rank $p<0.05$, Figure 3a). It is not predictive in ER- IDC patients nor in the cohort of all IDC patients (ER+ and ER-) (Data not shown). The relationship is not limited to breast cancer, as F/B is also a significant predictor of patient survival in Stage 1 colon adenocarcinoma patients (K-M Log Rank $p<0.05$, Figure 3b), but not predictive in stage 2 or higher patients, nor is it predictive in lung adenocarcinoma patients (Data not shown). These two results are significant because, in the two patient cohorts in which it is predictive, significant clinical decisions must be made: the primary tumor is removed in these patients but who gets, and does not get, adjuvant chemotherapy must be decided. In these patient cohorts there is a significant problem of "overtreatment" where patients often receive treatment who otherwise would not have metastasized. We believe that our data shows that SHG F/B is a rapid optical method to help further classify these patients and reduce overtreatment. We have filed a provisional patent on this work **(Provisional Patent Application 61/977,618)**.

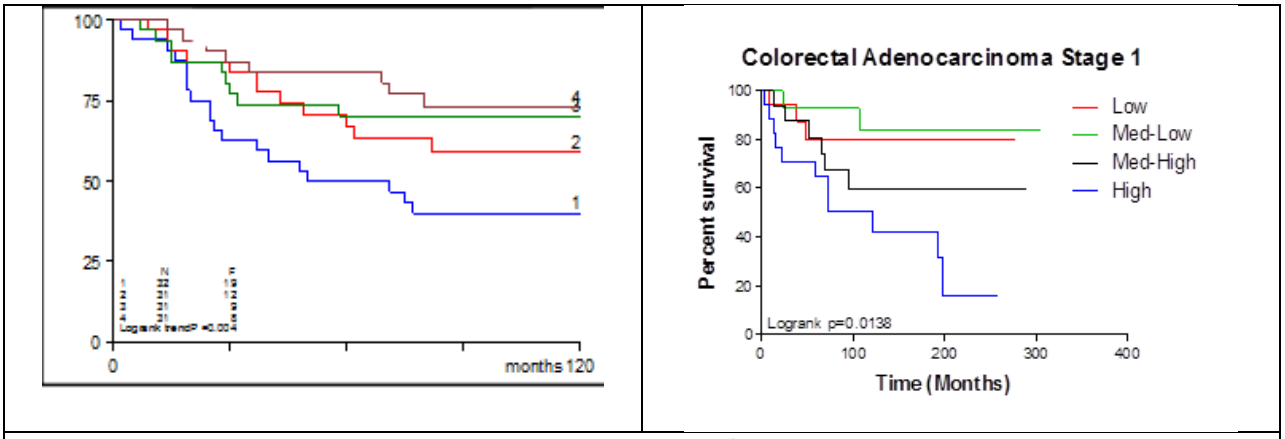


Figure 3. A. (left) Kaplan-Meier analysis reveals that SHG F/B is a significant indicator of metastasis free survival in Invasive Ductal Carcinoma (IDC) that has not metastasized to the sentinel lymph nodes (N0) upon presentation at the clinic. 221 patients were divided into four equally sized groups based upon the F/B of their primary tumor. B. (Right) K-M analysis of 69 patients reveals that F/B is also a significant indicator of overall survival in Stage 1 Colon Adenocarcinoma.

In parallel work we have been exploring the impact of emotional stress on breast tumor growth, first finding that the relevant molecular machinery, β-adrenergic receptor expression and function, is highly heterogeneous in different breast tumor cell lines **(Madden K, et al. 2011),** and that social

isolation was a functional model of emotional stress in mice and impacted tumor progression in breast tumor cell lines grown in the mammary fat pad **(Madden K, et al. 2013)**. We then found that pharmacological activation of the adrenergic receptor (AR) pathway altered tumor progression *in vivo* even in the absence of functional β-adrenergic receptors in the tumor cells themselves. Our aforementioned discovery that tumor associated macrophages influence SHG readouts of collagen ordering and metastatic output, and possibly operate via TNF-α, provided an unexpected candidate mechanism for this effect, and we found that AR stimulation does indeed alter collagen ordering as quantified by SHG **(Szpunar M, et al. 2013)**. This intersection of neuroscience and cancer, via the influence of the stromal compartment, has been extended by work revealing that administration of antidepressants affects the number of metastasis to the brain in a mouse model of brain metastatic breast cancer **(Shapovalov, et al. 2014)** although apparently through modulation of blood brain barrier permeability and not through the extracellular matrix of the brain parenchyma.

A cornerstone of our program to study the role of the extracellular matrix in tumor metastasis is to develop the optical techniques required for that study. Multiphoton Fluorescence Recovery After Photobleaching (MPFRAP) is a method to measure the diffusion coefficient of fluorescent tracer molecules with 3D resolution deep inside optically scattering tissue. The diffusion coefficient and size of a tracer molecule is an accurate reporter on the hindrance properties and pore sizes of the surrounding ECM. To apply this technique to the complex *in vivo* environment, with cell walls and other barriers to diffusion, we developed and demonstrated the theoretical framework necessary to perform MPFRAP in the presence of nearby barriers to diffusion of different geometries **(Sullivan et al., 2011)**. One must also take into account the presence of shear flow profiles, and we have developed the theoretical model to fit MPFRAP curves in the presence of shear flow, and tested that model in simulations and *in vitro* (Figure 4). We have also developed two novel methods that can measure the scattering directionality of second harmonic generation (the SHG "F/B ratio") in thick intact tissue, with a single image scan. This will be necessary for us to pursue our goal of quantifying matrix changes dynamically, in intact tumor models. The first method determines F/B by generating a series of backscattered images using a series of different sized confocal pinholes (Han et al., 2010). The second method takes an image pair using a "pinhole mirror" placed in the confocal plane. Backwards-propagating SHG passes through the pinhole and reaches one detector, while forward-propagating SHG diffuses down into the tissue, some returns

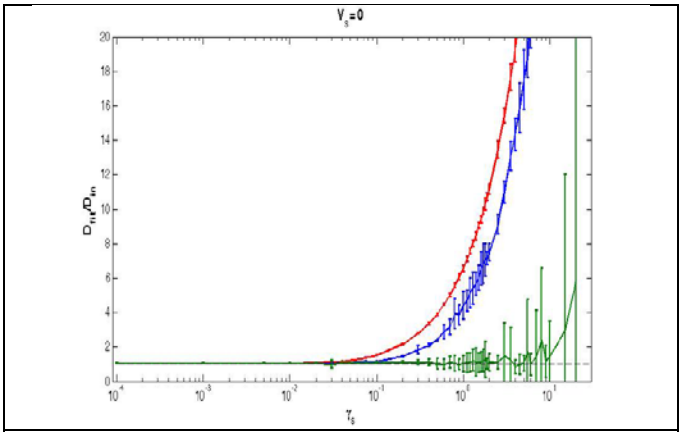
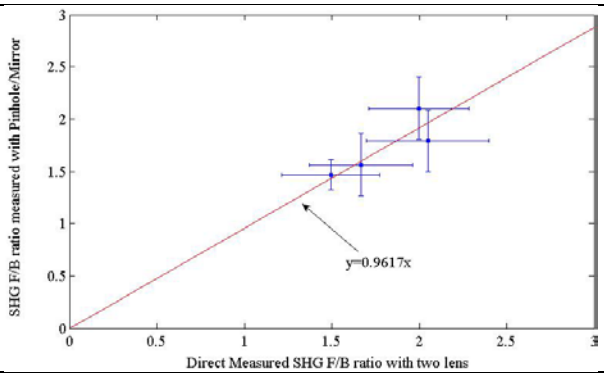


Figure 4. Results of Monte-Carlo simulations of MPFRAP recovery curves fitted with the classical MPFRAP model (red) which does not take into account flow, with an improved model of MPFRAP (blue) which accounts for simple flow (Sullivan et al., 2009), and with the most recent model (green) which accounts for shear flow. Accurate fits return a D_{fit} that is equal to D_{in} and hence appear as a value of one on this plot. For increasing shear rate (γ_s) each model begins to report incorrect diffusion coefficients, with the new model able to report correct values of D for significantly higher values of shear.

to the object plane via multiple scattering, reflects off of the mirror, and reaches a second detector. With suitable calibration F/B can be deduced (Figure 5). A provisional patent has been filed on this technique **(Provisional Patent Application 61/330,619).**

Figure 5. The new pinhole mirror method can measure SHG F/B in intact thick tissues. F/B was determined with the new method, then the samples were excised, thinly sliced, and F/B was also determined with classic two-detector direct measurement from thin samples. A slope of one indicates agreement between the two methods.



Finally, due to the work funded by this grant, the laboratory has developed significant expertise in the extracellular matrix, and optical methods for its study, and disseminated that expertise in a series of collaborative works **(Bouta et al. 2011, Chen et al. 2013, Arendt et al. 2013, Bouta et al. 2014)** and invited review articles **(Perry et al. 2012, Burke et al. 2014).**

Key Research Accomplishments:

1) Using second harmonic generation scattering directionality (F/B), which reports on collagen microstructure, we determined that tumor associated macrophages, stromal TNA-a, and stromal MMP-13 play a role in defining collagen microstructure and metastatic outcome.

2) In *in vitro* experiments we determined that collagen microstructure, as measured by F/B, directly influences tumor cell motility.

3) We discovered that F/B is significantly different in patient biopsy samples of different invasiveness and lymph node involvement. This led to the discovery that F/B is a significant predictor of metastasis free survival in ten-year follow up data in IDC N0 patients and stage 1 colon adenocarcinoma patients. These are two patient populations who suffer from “overtreatment” and for whom additional predictors of metastatic outcome are needed.

4) We expanded the applicability of the MP-FRAP technique to regions close to barriers to diffusion, and to regions with shear flow, and developed two novel techniques to measure SHG F/B in intact scattering tissue.

Conclusion

In conclusion we feel that we have successfully addressed the specific aims of the original proposal and made a significant contribution to the prevention of breast cancer mortality.

Publications, Abstracts, and Presentations:

Peer Reviewed Publications:

Sullivan K, **Brown E.** (2011) Diffusion and multi-photon fluorescence recovery after photobleaching in bounded systems. ***Physical Review E****.* 83(5): 051916 PMC3413246

Madden K, Szpunar M, **Brown E.** (2011) ß-adrenergic receptors regulate VEGF and IL-6 production by divergent pathways in high ß-AR-expressing breast cancer cell lines. ***Breast Cancer Research and Treatment.*** 130(3): 747-758 PMC3126869

Bouta E, Wood R, Perry S, **Brown E**, Ritchlin C, Xing L, Schwarz E. (2011) Measuring intranodal pressure and lymph viscosity to elucidate mechanisms of arthritic flare and therapeutic outcomes. ***Ann N Y Acad Sci.*** 1240(1):47-52. PMC3334848

Madden K, Szpunar M, **Brown E**. (2013) Early Impact of Social Isolation and Breast Tumor Progression in Mice. ***Brain Behavior and Immunity.*** 30 S135-S141. PMC3431437

Burke K, Tang P, **Brown E**. (2013) SHG Reveals Matrix Alterations During Breast Tumor Progression. ***J Biomed Optics.*** 18(3) 031106. PMC3595714

Arendt O, Schwaller B, **Brown E**, Eilers J, Schmidt H. (2013) Restricted diffusion of calretinin in cerebellar granule cell dendrites implies Ca^{2+} dependent interactions via its EF-hand 5 domain. ***Journal of Physiology****.* 591:3887-3899. PMC3764635

Burke R, Madden K, Perry S, Zettel M, **Brown E**. (2013) Tumor-associated macrophages and stromal TNF-α regulate collagen structure in breast tumor models as visualized by second harmonic generation. ***Journal of Biomedical Optics.*** 18(8):860003. PMC3731198

Perry S, Schueckler J, Burke K, Arcuri G, **Brown E**. (2013) Stromal Matrix Metalloprotease-13 knockout alters collagen I structure at the mammary tumor-host interface and increases lung metastasis. ***BMC Cancer.*** 13:411. PMC3766650

Szpunar M, Burke K, Dawes R, **Brown E**, Madden, K. (2013) The Antidepressant Desipramine and alpha2-Adrenergic Receptor Activation Promote Breast Tumor Progression in Association with Altered Collagen Structure. ***Cancer Prevention Research****. 6(12):1262-72.* PMC3862035

Chen T, Hilton M, **Brown E**, Zuscik M, Awad H. (2013) Engineering superficial zone features in tissue engineered cartilage. ***Biotechnology and Bioengineering.*** *110: 1476-1486.* PMC3694346

Bouta E, Wood R, **Brown E**, Rahimi H, Ritchlin C, Schwarz E. (2014) *In vivo* Quantification of Lymph Viscosity and Pressure in Lymphatic Vessels and Draining Lymph Nodes of Arthritic Joints in Mice. *Manuscript Accepted* ***J Physiol.*** PMC3961082

Shapovalov Y, Zettel M, Kelly E, Sipe G, Dickerson I, Majewska A*, and **Brown E***. (2014) Fluoxetine Modulates Breast Cancer Metastasis to the Brain. *Manuscript Accepted BMC Cancer*. PMC Journal – In Process

Invited Articles:

Perry S, Burke R, **Brown E**. (2012) Two Photon and Second Harmonic Microscopy in Clinical and Translational Cancer Research. *Invited Review* ***Annals of Biomedical Engineering.*** 40(2) 277-291***.*** PMC3342697

Burke K, Brown E. (2014) The Use of Second Harmonic Generation to Image the Extracellular Matrix During Tumor Progression. *Invited Perspective* ***Intravital*** *Manuscript Submitted.*

Sullivan K, Majewska A, **Brown E.** (2010) Single- and Two-Photon Fluorescence Recovery After Photobleaching. In: Yuste R (ed). Imaging: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. Chapter 42, pp. 655-666.

Madden K, Zettel M, Majewska A, **Brown E.** (2011) Imaging Tumors in the Brain. In: Helmchen F., Konnerth A. (eds). Optical Imaging Techniques: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. Chapter 91, pp. 1011-1017.

Perry S, Han X, **Brown E.** Second Harmonic Imaging of Tumors. In: Campagnola P, Pavone F (eds). Second Harmonic Generation Imaging. Taylor and Francis Press. *In Press.*

Abstracts (2011 and onwards):

Lapeira-Soto J, Madden K, **Brown E**. Multiphoton Microscopy Reveals Flawed Pro-Angiogenic Signaling in Breast Tumor Endothelial Cells. Biomedical Engineering Society Annual Meeting. 2011.

Madden K, Szpunar M, Bouta E, **Brown E**. Detection of sympathetic tyrosine hydroxylase-positive (TH+) nerve fibers in orthotopic mammary tumors by multiphoton laser scanning microscopy (MPLSM). Psychoneuroimmunology Research Society Annual Meeting. PNI Mechanisms of Disease: From Pathophysiology to Prevention and Treatment. 2011.

Madden K, Szpunar M, Byun D, Liverpool K, **Brown E**. Detection of Sympathetic Innervation and Norepinephrine in Orthotopic and Spontaneously Occurring Animal Models of Breast Cancer. AACR Special Conference on Tumor Microenvironmental Complexity: Emerging Roles in Cancer Therapy. 2011.

Szpunar M, Madden K, Liverpool K, **Brown E**. Sympathetic nervous system innervation and function in breast cancer models. AACR Special Conference on Tumor Microenvironmental Complexity. 2011.

Shapovalov Y, Sipe G, Zettel M, **Brown E**, Majewska A. Fluoxetine Enhances the Development of Breast Tumor Metastasis in the Brain. AACR Special Conference on Tumor Microenvironmental Complexity. 2011.

Szpunar M, Madden K, Liverpool K, **Brown E**. Sympathetic nervous system innervation and function in a beta-adrenergic receptor negative breast cancer model. Psychoneuroimmunology Research Society. 2011

Szpunar M, Madden K, Liverpool K, **Brown E**. Sympathetic nervous system innervation and function in breast cancer models. DoD Breast Cancer Research Program Era of Hope Conference. 2011. *Student Poster contest finalist.*

Madden K, Szpunar M, Liverpool K, **Brown E**. Sympathetic Nerves in Breast Cancer: Function and In Vivo Imaging in an Orthotopic Animal Model. DoD Breast Cancer Research Program Era of Hope Conference. 2011.

Burke R, Perry S, Madden K, **Brown E**. Understanding Collagen Ordering to Predict and Prevent Metastasis. DoD Breast Cancer Research Program Era of Hope Conference. 2011.

Han X, Lapeira-Soto J, Sullivan K, Szpunar M, Madden K, **Brown E**. Angiogenic Signaling in Living Breast Tumor Models. DoD Breast Cancer Research Program Era of Hope Conference. 2011.

Majewska A, Shapovalov Y, Zettel M, Sipe G, **Brown E**. Brain Plasticity and Its Effects on Breast Tumor Metastasis to the Brain. DoD Breast Cancer Research Program Era of Hope Conference. 2011.

Majewska A, Shapovalov Y, Zettel M, Cash S, **Brown E**. The Influence of Neuronal Activity on Breast Tumor Metastasis to the Brain. DoD BCRP Era of Hope Conference. 2011.

Lapeira-Soto J, Madden K, **Brown E**. Abnormal VEGF_induced Ca2+ Signalling in Purified Tumor Endothelial Cells. Biomedical Engineering Society Annual Meeting. 2012

Lapeira-Soto J, Perry S, O'Connell P, Brown E, and **Brown E**. Expanding the applicability of multi-photon fluorescence recovery after photobleaching by incorporating shear stress in a laminar flow model. Presentation, Biomedical Engineering Society Annual Meeting. 2012

Szpunar M, Madden K, Burke K, Byun D, Liverpool K, **Brown E**. Evidence for Sympathetic Nervous System Modulation of Mammary Tumor Pathogenesis via Tumor Collagen. Psychoneuroimmunology Research Society Annual Meeting. 2012.

Burke K. and **Brown E**. Using SHG to Study Breast Cancer Tumor Progression. The Engineering in Medicine and Biology Conference. 2012.

Burke K. and **Brown E**. Using SHG to Study Breast Cancer Tumor Progression. American Association of Cancer Research. 2013.

Dawes R, Burke K, Stastka P, Madden K, **Brown E**.The neurotransmitter norepinephrine and ß2-AR activation of MB-231 breast cancer cells alters fibrillar collagen structure. American Association of Cancer Research: Advances in Breast Cancer Research. 2013

Bouta E, Wood R, **Brown E**, Rahimi H, Ritchlin C, Schwarz E. Pressure and Viscosity Measurements in Afferent Lymphatics to Elucidate the Mechanisms of Arthritic Flare. Vascular Biology and Microcircultion Society Meeting, 2013.

Shapovalov Y, Amico-Ruvio S, Spielman S, Lamantia C, **Brown E**, Majewska A. " Analysis of glial activation in a murine model of brain metastatic breast cancer". American Association of Cancer Research: Advances in Breast Cancer Research. 2013

Patents, Patent Applications:

Han XX, **Brown E** (2010) Epidetection method and apparatus for measuring the ratio of forward-propagating to back-propagating second harmonic signal. Provisional Patent Application 61/330,619.

Brown E, Perry W, Burke K, Kottman R, Sime P, Sharp J. (2014) Method and apparatus to diagnose metastatic and progressive potential of cancer, fibrosis, and other diseases. Provisional Patent Application 61/977,618

Reportable Outcomes: N/A

Other Achievements:

Promotion to Associate Professor with tenure, May 2011. Secured transition from Medical Center appointment to Main Campus (9 month) appointment, 2013. Appointment as Scientific Director, University of Rochester Multiphoton Microscopy Core Facility, July 2011. Appointment to Integration Panel, Department of Defense Ovarian Cancer Research Program, 2013. 3 graduate students have secured individual training grants while working on these projects (2 DoD BCRP, 1 NIH). 5 graduate students who have contributed to these results have earned their Ph.D. degrees.

Appendix 1. List of personnel receiving pay from the research effort:

Ed Brown	Sarah Sushchyk
Kelley Madden	Katie Cooley
Avraham Salzman	Seth Perry
Khawarl Liverpool	Jill Kulla
Kelley Sullivan	Giuseppe Arcuri
Javier Lapeira	Erin Keegan
Edith Lord	Peter O'Connell
Ping Tang	Jill Schueckler
Dan Byun	Jesse Sharp
Martha Zettel	Petr Stastka
Ryan Burke	Zhou Xu
Kelly Kyker-Snowman	Ryan Dawes
Kathryn Fitzgerald	Mehar Cheema
Kattie Litts	